

5100 Paint Branch Parkway  
College Park, MD 20740-3835

M-a-85 (Revision #11)

March 10, 2004

TO: All Regional Food and Drug Directors  
Attn: Regional Milk Specialists

FROM: Milk Safety Branch (HFS-626)

SUBJECT: Beta lactam Test Methods For Use Under Appendix N And Section 6 Of The  
Grade "A" Pasteurized Milk Ordinance (PMO)

This coded memorandum replaces and rescinds the previous revision of this coded memorandum (M-a-85, Revision #10).

This revision is based on actions taken by the National Conference on Interstate Milk Shipments' (NCIMS) Laboratory Committee, addressing the acceptance of the DSM Food Specialties' Delvo®Scan Reader as an option for use with the Delvotest® P 5 PACK test kit at their committee meeting held in Reno, Nevada in November 2003 and the phasing out of two (2) tests.

The individual Test Tables presented in this revision provide data points that were derived from testing at least thirty (30) samples at each concentration for each drug detected.

This revision modifies the Tables to reflect the addition of the Delvo®Scan Test Reader as an option for use with the Delvotest® P 5 PACK; the removal of the Delvo-X-Press, as this test is no longer being manufactured; and the removal of the Charm II Tablet Transit, as this test is no longer in use. It also lists the last date (July 7, 2004) that the IDEXX SNAP test kit can be used as the manufacturer has stopped production of this kit and is only manufacturing the New SNAP test kit. For information regarding these tests please refer to M-a-85 (Revision #10) to see how these tests performed.

The attached information is summarized from the evaluation of data submitted by test sponsors. Information related to the protocol used in this evaluation is available from Dr. Linda D. Youngman, FDA's Center for Veterinary Medicine (CVM), (301) 827-8010. Additional information regarding performance of these tests may be available from the test kit manufacturers.

Label claims for these tests were evaluated for use on raw, commingled bovine milk samples. The evaluation protocol did not measure the performance of these tests in the assay of drug residues in other milk matrices, i.e., pasteurized milk or milk taken from individual cows, although claims for such use are made by some of the manufacturers of these tests.

**NOTE:** FDA recognizes that six (6) Beta lactams are widely used in treating disease in lactating dairy cattle and are the most likely to cause a residue in milk if misused. These are penicillin, ceftiofur, cloxacillin, cephalixin, amoxicillin, and ampicillin. While it is preferred that monitoring for Beta lactams include all of these drugs, at this time, the Agency is recommending that methods be utilized that have been shown to detect at least four (4) of the six (6) Beta lactams identified above.

Testing for drug residue(s) in compliance with the provisions of Sections 6 and 7 of the PMO may be accomplished by the use of any accepted Appendix N test for raw milk or an accepted Section 6 test for raw and pasteurized milk.

The NCIMS Executive Board has agreed that future updates to M-a-85 that add, delete or revise these tests will not require a public comment period or follow the protocol established in the Procedures document for the issuance of M-a's.

Copies of this coded memorandum are enclosed for distribution to Regional Milk Specialists, State Milk Regulatory Agencies, State Laboratory Evaluation Officers and State Milk Sanitation Rating Officers in your region. This memorandum should be widely distributed to representatives of the dairy industry, State Veterinarians, State Veterinary and Pharmacy Boards, Veterinarian Professional Organizations and other interested parties and also will be available on the FDA Web site at <http://www.cfsan.fda.gov> at a later date.

If you would like an electronic version of this document prior to it being available on the CFSAN Web Site, please e-mail your request to [Robert.Hennes@cfsan.fda.gov](mailto:Robert.Hennes@cfsan.fda.gov).

/ss/

Larry Maturin, PhD, Chief  
Laboratory Quality Assurance Team

/ss/

Robert F. Hennes, RS, MPH, Chief  
Milk Safety Team

**ATTACHMENT TO M-a-85 (REVISION #11)<sup>1</sup>**

**MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS**

**BETA lactams**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE OR SAFE LEVEL	10 ppb	10 ppb	50 ppb <sup>2</sup>	20 ppb	10 ppb	5 ppb
<b>SCREENING TEST</b>						
CHARM <i>B. stearothermophilus</i> TABLET DISK ASSAY <sup>3, 4, 5</sup>	7.5	6.7	75 <sup>6</sup>	11.7	48 <sup>6</sup>	3.8
CHARM II TABLET BETA lactam TEST (COMPETITIVE ASSAY) <sup>3</sup>	7.5	5.7	7.1	4.2	70 <sup>6</sup>	3.0
CHARM II TABLET BETA lactam TEST (SEQUENTIAL ASSAY)	8.1	6.6	11.3	4.1	50 <sup>6</sup>	3.4
CHARM II TABLET BETA lactam TEST (QUANTITATIVE ASSAY) <sup>7</sup>	8.1	6.6	11.3	4.1	8.5	3.4
CHARM II TEST FOR CLOXACILLIN IN MILK (COMPETITIVE ASSAY) <sup>3, 8</sup>	ND <sup>9</sup>	ND <sup>9</sup>	ND <sup>9</sup>	ND <sup>9</sup>	8.5	ND <sup>9</sup>
CHARM SL BETA lactam TEST <sup>10</sup>	5.6	8.5	46	13.7	50 <sup>6</sup>	3.6
CHARM SL6 <sup>TM</sup> BETA Lactam TEST	7.1	9.6	37.5	18.7	8.3	4.2
DELVOTEST P 5 PACK (READER) <sup>3, 11</sup>	4.6	4.0	NA <sup>12</sup>	8.2	NA <sup>12</sup>	2.1
DELVOTEST P 5 PACK (VISUAL) <sup>3, 4, 5, 13</sup>	4.6	4.0	NA <sup>12</sup>	8.2	NA <sup>12</sup>	2.1
DELVOTEST P/DELVOTEST P MINI <sup>4</sup>	7.7	5.1	>50	7.0	30 <sup>6</sup>	3.1
DELVOTEST SP/DELVOTEST SP MINI	6.0	7.9	>50	7.7	33 <sup>6</sup>	2.7
PENZYME MILK TEST <sup>4</sup>	6.0	7.0	80 <sup>6</sup>	11.6	80 <sup>6</sup>	5.0
PENZYME III TEST PROCEDURE <sup>4</sup>	5.3	5.6	NA <sup>12</sup>	14.3	NA <sup>12</sup>	4.3
SNAP BETA lactam TEST KIT (READER) <sup>3, 14</sup>	8	8.2	10.5	3.5	50 <sup>6</sup>	3.5
SNAP BETA lactam TEST KIT (VISUAL) <sup>3, 4, 14</sup>	9.4	8.2	9.6	3.0	50 <sup>6</sup>	3.4
New SNAP BETA lactam TEST KIT <sup>14</sup>	7.3	5.8	5.4	11.7	50 <sup>6</sup>	3.0
PARALLUX BETA lactam ASSAY	3.6 <sup>15</sup>	2.9 <sup>15</sup>	33.7	16.3	7.4	3.2 <sup>15</sup>

Continued on next page

<p><b>CONTINUED: MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS<sup>1</sup></b></p> <p><b>BETA lactams</b></p>
---

**FOOTNOTES:**

1. Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following tables and should be considered when selecting drug residue monitoring tests. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.
2. Parent Drug
3. This test is acceptable for use to detect Beta lactam residues when used with bovine pasteurized whole and skim milk.
4. Refer to M-I-01-4 for certification requirements to use this visual test.
5. This test is acceptable for testing raw, commingled goat milk.
6. 90/95% concentrations were not determined for sensitivities significantly above the tolerance/safe level.
7. Test sensitivity when presumptive positive milk samples are verified in accordance with label directions using the Charm II Tablet Sequential Assay and the Charm II Test for Cloxacillin in Milk.
8. For Appendix N bulk milk tanker screening, this test must be used in combination with other approved screening methods in order to detect at least four (4) of the six (6) targeted Beta lactam drugs.
9. ND indicates "Not Detected".
10. The Charm SL Beta lactam Test is acceptable for testing raw, commingled goat milk (M-I-03-3).
11. The DelvoScan Reader option for the Delvotest 5 P Pack has not been validated in fat-free chocolate, whole chocolate, half & half, heavy cream and pasteurized goat milk.
12. NA indicates "Data Not Available".
13. The Delvotest 5 P Pack (VISUAL) is acceptable to detect ampicillin, amoxicillin, cephalixin and penicillin residues in bovine fat-free chocolate, whole chocolate, half & half, and heavy cream and pasteurized goat milk.
14. The SNAP Beta lactam Test is not longer being manufactured and will not be acceptable for use after July 2, 2004. The visual reading option is not available with the New SNAP.
15. For Parallax tests, due to significant cross reactivity of cloxacillin in the Cillins channel, a positive Cillins test may be due to cloxacillin.

**CHARM *B. stearotheophilus* TABLET DISK ASSAY**  
**DRUG CONCENTRATION RESPONSE**<sup>1,2</sup>

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				0
2	0	0	0	0
3				0
4	10	3	0	55
5				100
6	30	67		
8	90	100	0	
10	100	100		
14			100	
20			100	

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

**CHARM II TABLET BETA lactam TEST (COMPETITIVE ASSAY)**  
**DRUG CONCENTRATION RESPONSE**<sup>1,2</sup>

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>3</sup>	20	5
DRUG CONCENTRATION (ppb)					
1					10
2	3	3		30	67
3					97
4	10	43		100	100
5			60		100
6	83	97			
8	100	100		100	
10	100	100	100		
14				100	
20			100	100	
50			100		

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> Parent drug

**CHARM II TABLET BETA lactam TEST (SEQUENTIAL ASSAY)**  
**DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>3</sup>	20	5
DRUG CONCENTRATION (ppb)					
1					0
2	0	0		3	10
3					80
4	20	10		100	100
5			0		100
6	23	83			
8	93	97		100	
10	100	100	83		
14				100	
20			100	100	
50			100		

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> Parent drug

**CHARM II TABLET BETA lactam TEST (QUANTITATIVE ASSAY)**  
**DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXICILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>3</sup>	20	10	5
DRUG CONCENTRATION (ppb)						
1						0
2	0	0		3	0	10
3						80
4	20	10		100	3	100
5			0			100
6	23	83			17	
8	93	97		100	87	
10	100	100	83		100	
14				100		
20			100	100		
50			100			

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> Parent drug

**CHARM II TEST FOR CLOXACILLIN (COMPETITIVE ASSAY)  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	CLOXACILLIN
TOLERANCE/SAFE LEVEL (ppb)	10
DRUG CONCENTRATION (ppb)	
2	0
4	3
6	17
8	87
10	100

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

**CHARM SL BETA lactam TEST  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>3</sup>	20	5
DRUG CONCENTRATION (ppb)					
1					0
2	3	3			13
3					73
4	70	13		0	100
5					100
6	100	83			
8	100	100		50	
10	100	97	0		
12				97	
16				100	
20			0	100	
30			13		
40			70		
50			100		

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> Parent drug

**CHARM SL6™ BETA lactam TEST  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>3</sup>	20	10	5
DRUG CONCENTRATION (ppb)						
1						7
2	0	0			0	23
3						93
4	7	3		0	40	100
5						100
6	57	17			83	
8	97	77		7	97	
10	100	100	0		100	
12				53		
16				100		
20			27	100		
30			73			
40			93			
50			100			

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> Parent drug

**DELVOTEST P 5 PACK (VISUAL AND READER)  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				3
2	10	7	3	60
3				100
4	100	97	100	100
5				100
6	100	100		
8	100	100	100	
10	100	100		
14			100	
20			100	

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration



**DELVOTEST P/DELVOTEST P MINI  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				0
2	33	3	0	0
3				100
4	47	70	7	100
5				100
6	93	100		
8	97	100	100	
10	100	97		
14			100	
20			100	

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

**DELVOTEST SP/DELVOTEST SP MINI  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				7
2	0	0	3	47
3				100
4	80	10	17	100
5		33		100
6	100			
8	100	100	100	
10	97	100		
14			100	
20			100	

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

**PENZYME MILK TEST  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				0
2	10	3	7	0
3				33
4	97	80	83	97
5				100
6	100	100		
8	100	100	100	
10	100	100		
14			100	
20			100	

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

**PENZYME III TEST PROCEDURE  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				0
2	7	43	10	33
3				77
4	77	97	87	97
5				100
6	100	100		
8	100	100	100	
10	100	100		
12			100	
20			100	

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

**SNAP BETA lactam TEST KIT (READER)  
DRUG CONCENTRATION RESPONSE<sup>1,2,3</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>4</sup>	20	5
DRUG CONCENTRATION (ppb)					
1				10	7
2				93	80
3	0	30		100	100
4	3	93	90	100	97
5				100	100
6	90	100	100		
8	100	100	100		
10	100	100	100		
12			100		

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> The SNAP BETA lactam TEST is being replaced by the New SNAP BETA lactam TEST and cannot be used after July 2, 2004.

<sup>4</sup> Parent drug

**SNAP BETA lactam TEST KIT (VISUAL)  
DRUG CONCENTRATION RESPONSE<sup>1,2,3</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>4</sup>	20	5
DRUG CONCENTRATION (ppb)					
1				20	7
2		27		100	80
3	0			100	100
4	0	90	77	100	97
5				100	100
6	40	100	100		
8	80	100	100		
10	100	100	100		
12			100		

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> The SNAP BETA lactam TEST is being replaced by the New SNAP BETA lactam TEST and cannot be used after July 2, 2004. The visual reading option is not available with the New SNAP.

<sup>4</sup> Parent drug

**NEW SNAP BETA lactam TEST KIT  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>3</sup>	20	5
DRUG CONCENTRATION (ppb)					
1					7
2	0	0		0	37
3					93
4	20	37		0	100
5					100
6	70	100			
8	100	100		0	
10	100	100	87		
12				100	
20			100	100	
30			100		
50			100		

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> Parent drug

**PARALLUX BETA lactam ASSAY  
DRUG CONCENTRATION RESPONSE <sup>1,2</sup>**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 <sup>3</sup>	20	10	5
DRUG CONCENTRATION (ppb)						
1						3
2	30	70		0	0	20
3						87
4	100	100		0	0	100
5			0			100
6	100	100			60	
7						
8	100	100		3	100	
9						
10	100	100	3		100	
12				30		
20			33	100		
30			93			
50			100			

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

<sup>3</sup> Parent drug

# **MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS <sup>1</sup>**

## **SULFONAMIDES**

### **CHARM II SULFA DRUG TEST (COMPETITIVE ASSAY)<sup>1</sup>**

<b>SCREENING TEST</b>	<b>SULFADIMETHOXINE</b>	<b>SULFAMETHAZINE</b>	<b>SULFATHIAZOLE</b>	<b>SULFADIAZINE</b>
CHARM II SULFA DRUG TEST (COMPETITIVE ASSAY)	4.0	9.4	7.3	4.9

<sup>1</sup> Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following table. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.

### **CHARM II SULFA DRUG TEST (COMPETITIVE ASSAY) <sup>1,2</sup>**

<b>DRUG</b>	<b>SULFADIMETHOXINE</b>	<b>SULFAMETHAZINE</b>	<b>SULFATHIAZOLE</b>	<b>SULFADIAZINE</b>
TOLERANCE/SAFE LEVEL (ppb)	10	10	10	10
DRUG CONCENTRATION (PPB)				
2	97	7	0	40
4	100	80	57	100
6	100	97	100	100
8	100	100	100	100
10	100	100	100	97

<sup>1</sup> Percent positive

<sup>2</sup> Based on 30 samples at each concentration

**MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS <sup>1</sup>**  
**TETRACYCLINES**

**CHARM II TETRACYCLINE DRUG TEST (COMPETITIVE)**  
**90/95 PERCENT DETECTION LEVELS (ppb)**

DRUG	90/95% DETECTION LEVELS (ppb)
Chlortetracycline	257
Oxytetracycline	119
Tetracycline	67

<sup>1</sup> Parts per billion (ppb), which can be, detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following tables and should be considered when selecting drug residue monitoring tests. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.

**CHARM II TETRACYCLINE DRUG TEST (COMPETITIVE)**

DRUG	Chlortetracycline	Oxytetracycline	Tetracycline
TOLERANCE/SAFE LEVEL (ppb)	300 ppb (Chlortetracycline + Tetracycline + Oxytetracycline)		
DRUG CONCENTRATION (ppb)			
20			0
30			7
40			37
60		13	93
70		37	
90	17		
100		87	
120	20		
150		100	
160	77		
230	93		
300	97	100	100